

The mode of action of urogenital pathogens is now better understood and involves formation of biofilms in the intestine. Intestinal biofilms then become a reservoir for urogenital pathogens which invade the urogenital tract, where more biofilms are formed. Urogenital tract biofilms then become the reservoir for

Previous studies have shown that specially selected probiotic lactobacilli, provided in a pessary inserted into the vagina, can colonize (Reid, et al. 1994) and compete against colonization of enterococci and other uropathogens (Bruce & Reid, 1998). The art also describes the use of *Lactobacillus* to prevent and treat urinary and urogenital infections. There is therefore a distinct need for combinations of probiotic organisms which confer vaginal health benefits to the host.

15           The present invention demonstrates specially  
selected lactobacilli with antagonistic properties  
against urogenital pathogens, can colonize the vagina and  
provide protection against infection after oral intake.  
The present invention, for the first time, establishes  
20 that oral intake of *Lactobacillus* can successfully  
deliver probiotic therapy to women in need thereof.

The present invention provides methods and compositions for the treatment and inhibition of urogenital infection caused by pathogenic organisms.

25 Oral administration of one or more *Lactobacillus*, other probiotic compounds in a pharmaceutically acceptable or food grade carrier, such as milk or portions thereof, including yogurt, provide a safe and effective means for colonizing the intestine, urinary tract and vagina and

30 treating, inhibiting or reducing the occurrence of urogenital infections.

In the practice of the compositions and methods of the present invention, the *Lactobacillus* may be

5 *paracasei*, *L. jensenii*, *L. gasseri*, *L. reuteri*, *L.*  
*cellobiosus*, *L. brevis*, *L. delbrueckii*, *L. rogosae* and *L.*  
*bifidum*.

In one embodiment of the present invention a method is provided for establishing a healthy gastrointestinal and urogenital flora in females throughout life comprising orally administering a therapeutically effective amount of at least one *Lactobacillus* and a pharmaceutically acceptable carrier. In another embodiment, a therapeutically effective amount of two lactobacilli is administered. In a preferred embodiment a therapeutically effective amount of a combination of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 is administered. In a further embodiment of the method a therapeutically effective amount of a second probiotic organism is administered. Bifidobacteria is the preferred second probiotic organism. The Bifidobacterium is preferably selected from the group consisting of *B. bifidum*, *B. breve*, *B. adolescentis*, or *B. longum*.

In still another embodiment, the present invention provides a method for inhibiting, treating or

reducing the occurrence of urogenital infections in a mammal in need of such treatment by oral administration of at least one *Lactobacillus* and other probiotic organisms. In a preferred embodiment, the probiotic  
5 organism is *Bifidobacterium*.

In still yet another embodiment, the present invention describes a method for inhibiting urogenital pathogen colonization of the gastrointestinal and urogenital tract in mammals. In a preferred embodiment,  
10 the mammals are humans. In another embodiment, the urogenital pathogens are *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Providencia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Bacteroides* spp., *Mobiluncus* spp. *Trichomonas* spp. *Fusobacterium*  
15 spp., *Enterococcus* spp., *Gardnerella* spp. and/or yeast.

In a further embodiment, the present invention describes a method for maintaining healthy urogenital flora by oral intake of *Lactobacillus*.

In a most preferred embodiment, the  
20 *Lactobacillus* species are *L. rhamnosus* GR-1 (ATCC 55826), *L. fermentum* RC-14 (ATCC 55845) and *L. fermentum* B-54 (ATCC 55884).

In another embodiment, the present invention provides a method for preventing or reducing the biofilm  
25 load of urogenital pathogens in the intestine, vagina, perineum and bladder in a mammal in need of such treatment by oral administration of *Lactobacillus*, anti-urogenital pathogen probiotics together with a suitable carrier.

30 In still another embodiment, the present invention provides a method of improving vaginal health by oral intake of at least one *Lactobacillus*.

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In still yet another embodiment, the present invention provides a method of treating vaginitis by oral intake of at least one *Lactobacillus*.

In one embodiment, the present invention  
5 provides a method of treating bacterial vaginosis by oral intake of at least one *Lactobacillus*.

In another embodiment, the present invention provides a probe for the detection of lactobacilli in a biological sample.

10 In a preferred embodiment, the suitable carrier is milk or portions thereof, including yogurt and other such foods, including, but not limited to, milk shakes and powdered milk products; non-milk products and non-lactose containing products, including calcium carbonate.

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#### **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a pie chart demonstrating the survival and colonization of *L. rhamnosus* GR-1, *L. fermentum* RC-14 and *L. fermentum* B-54 following oral  
20 ingestion in the intestinal tract, as measured in a Day 7 stool sample, from a patient with recurrent urogenital infections. This demonstrates safe passage of probiotic *Lactobacillus* through the stomach and intestine.

Figure 2 is a pie chart demonstrating the  
25 survival and colonization by *L. fermentum* RC-14 following oral ingestion in the intestinal tract, as measured in a Day 14 stool sample, from a patient with recurrent urogenital infections. This also demonstrates safe passage through the stomach and intestine and ability of  
30 lactobacillus to ascend into the urogenital tract.

Figure 3 is a schematic depicting the process of urinary tract and vaginal infection.

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Figure 4 is a schematic depicting the effect of lactobacillus ingestion on urogenital pathogens in the intestine and vagina.

Figure 5 is a schematic depicting the effect of lactobacillus treatment for urinary tract infection.

Figure 6 is a polyacrylamide gel electrophoresis showing PCR products identified. Lane 1- *L. rham.* ATCC 7469; Lane 2- *L. rham.* GR-1; Lane 3- *L. rham* C3-A; Lane 4- *L. casei ssp. casei* ATCC 393; Lane 5- *L. Para. ssp. para.* ATCC 25302; Lane 6- *L. plant.* ATCC 14917; Lane 7- *L. ferm.* ATCC 14931; Lane 8- *L. ferm.* ATCC 23271; Lane 9- *L. ferm.* ATCC 8289; Lane 10- *L. ferm.* ATCC 11739; Lane 11- *L. ferm.* ATCC 14932; Lane 12- *L. ferm.* RC14 (1 band); Lane 13- (*L. ferm.* B54 has the same ribotype as RC14) (1 band); Lane 14- *L. acid.* ATCC 4356; Lane 15- *L. jensenii* ATCC 25258.

Figure 7A is a bar graph showing the effect of the combination of Lactobacillus GR-1/RC-14 on vaginal flora over a period of four weeks.

Figure 7B is a bar graph showing the effect of the combination of Lactobacillus GR-1/RC-14 on total vaginal yeast and total vaginal Lactobacillus counts in 64 healthy women.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to methods and compositions for maintaining the health of the urogenital tract, and for treating, inhibiting or reducing the occurrence of urogenital infections in mammals by oral administration of one or more *Lactobacillus* strains alone or in combination with other probiotic organisms together with a pharmaceutically acceptable carrier. In a preferred embodiment, the present invention contemplates the oral intake of *L.*

*fermentum* RC-14 and *L. rhamnosus* GR-1 in combination with each other, together with a pharmaceutically acceptable carrier. As defined by the present invention, a "probiotic" compound is a mono or mixed culture of

5 microorganisms which, when ingested by a mammal, for example a human, affects the host beneficially. A preferred probiotic compound is *Bifidobacterium*.

Lactobacilli which can be orally administered using the method described in the present invention may

10 be administered as viable whole cells. The *Lactobacillus* may be aerobically or microaerophillically grown and selected from *L. rhamnosus*, *L. acidophilus*, *L. crispatus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. paracasei*, *L. jensenii*, *L. gasseri*, *L. reuteri*, *L. cellobiosis*, *L. brevis*, *L. delbrueckii*, *L. rogosae* and *L. bifidum*. In a

15 preferred embodiment, the *Lactobacillus* species are *L. rhamnosus* GR-1 (ATCC 55826), *L. fermentum* RC-14 (ATCC 55845) and *L. fermentum* B-54 (ATCC 55884).

In accordance with the present invention,

20 orally administered *Lactobacillus* species can colonize the human intestinal and genital tracts and urethra thereby competitively inhibiting and otherwise disrupting or interfering with colonization of urogenital pathogens into biofilms. The orally administered *Lactobacillus*

25 species can also stimulate the indigenous normal flora of the urogenital tract thereby preventing, treating and/or reducing the occurrence of infections caused by urogenital pathogens. The urogenital pathogens inhibited and otherwise depleted by the *Lactobacillus* of the

30 present invention include, but are not limited to, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Providencia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Bacteroides* spp., *Mobiluncus* spp.

*Trichomonas* spp. *Fusobacterium* spp., *Enterococcus* spp., *Gardnerella* spp. and yeast.

In accordance with the present invention, following diminuation of the pathogenic biofilms in the  
5 intestinal, genital and urinary tracts, the orally administered *Lactobacillus* of the present invention can maintain healthy urogenital flora. By "healthy urogenital flora" is meant a total lactobacilli count greater than 10,000 more colony forming units of  
10 *Lactobacillus* than Gram negative rods, yeast and Gram positive cocci. By "diminuation of pathogenic biofilms" is meant flora dominated by lactobacilli with no adherent pathogenic microorganisms (e.g. *Enterococcus faecalis*) on bladder uroepithelial cells, as measured by conventional  
15 urinalysis, or depleted numbers of pathogenic microorganisms (to less than 10 per cell) on vaginal cells.

Also defined within the present invention are compositions suitable for establishing, maintaining or  
20 restoring a healthy gastrointestinal and urogenital flora in females throughout life which comprise one or more *Lactobacillus* viable whole cells, non-viable whole cells or cell wall fragments and a pharmaceutically acceptable carrier. By "throughout life" is meant in the neonatal  
25 period, during childhood and in the pre-menopausal and post-menopausal periods. By "healthy gastrointestinal and urogenital flora" is meant flora that is predominantly colonized by non-pathogenic organisms and where there are no signs or symptoms of infection or  
30 disease. In a preferred aspect, the compositions of the present invention contain *L. fermentum* RC-14 and *L. rhamnosus* GR-1.



In another preferred aspect, the *Lactobacillus* is aerobically, microaerophilically or anaerobically grown and may be selected from the group consisting of *Lactobacillus casei*, *L. acidophilus*, *L. plantarum*, *L.*

5 *fermentum*, *L. brevis*, *L. jensenii*, *L. crispatus*, *L. rhamnosus*, *L. reuteri*, *L. paracasei*, *L. gasseri*, *L. cellobiosus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. collinoides*, *L. buchneri*, *L. rogosae* and *L. bifidum*.

10 The *Lactobacillus* may be microaerophilically or anaerobically grown and selected from the group consisting of *Lactobacillus rhamnosus* (GR-1 (ATCC 55826), *L. rhamnosus* GR-2 (ATCC 55915), *L. rhamnosus* GR-3 (ATCC 55917), *L. rhamnosus* GR-4 (ATCC 55916), *L. rhamnosus* RC-  
15 9, *L. rhamnosus* RC-17 (ATCC 55825), *L. casei* var *alactosus* RC-21, *L. casei* NRC 430, *L. casei* ATCC 7469, *L. rhamnosus* 81, *L. rhamnosus* 76, *L. rhamnosus* 36, *L. casei* RC-65, *L. casei* RC-15, *L. casei* 558, *L. casei*, RC-21, *L. casei* 55, *L. casei* 8, *L. casei* 43, *L. plantarum* RC-12  
20 (ATCC 55895), *L. acidophilus* RC-25, *L. plantarum* RC-19, *L. jensenii* RC-11 (ATCC 55901), *L. acidophilus* ATCC 4357, *L. acidophilus* 2099 B, *L. acidophilus* 2155C, *L. acidophilus* T-13, *L. acidophilus* 1807B, *L. acidophilus* RC-16, *L. acidophilus* RC-26, *L. acidophilus* RC-10, *L.*  
25 *acidophilus* RC-24, *L. acidophilus* RC-13, *L. fermentum* RC-14, *L. acidophilus* RC-12, *L. acidophilus* PTL19, *L. acidophilus* RC-22, *L. acidophilus* 2099B, *L. acidophilus* 2155C, *L. acidophilus* T-13, *L. plantarum* ATCC 8014, *L. plantarum* UH 2153, *L. plantarum* 260, *L. plantarum* RC-20,  
30 *L. plantarum* 75, *L. plantarum* RC-6, *L. fermentum* A-60, *L. fermentum* B-54 (ATCC 55920), *L. fermentum* RC-14, *L. cellobiosus* RC-2, *L. crispatus* 1350B, *L. crispatus* PTL37, and *L. crispatus* 2142B.

In a further embodiment, the present invention describes a method of administering probiotic organisms orally for restoring a healthy urogenital and intestinal flora over the various life cycle stages of women  
5 including pregnancy and post-menopause, wherein the pathogenic flora is dominated by *Mobiluncus*, *Gardnerella*, *Bacteroides*, *Fusobacterium*, *Prevotella*, *Peptostreptococcus*, *Porphyromonas*, *Mycoplasma* or group B streptococci, or *Escherichia coli*, *Enterococcus sp*,  
10 *Klebsiella sp*, *Pseudomonas sp*, *Streptococcus sp*, *Proteus sp*, and other pathogens which cause urinary tract infections, and yeast including *Candida albicans*, for example.

In still another embodiment, the present  
15 invention describes a method of administering probiotic organisms orally for reducing and inhibiting candida colonization, bacterial vaginosis and yeast vaginitis.

The *Lactobacillus* useful in accordance with the practice of the present invention preferably attaches to  
20 human epithelial cells to a level of about 10 to 165 organisms per cell by hydrophobic, hydrophilic or other adhesion interactions.

In another embodiment, the present invention provides a method for selecting lactobacilli and  
25 bifidobacteria useful for improving urogenital health. Criteria are provided herein for characterizing a selected *Lactobacillus* or *Bifidobacterium* as candidates for the contemplated methods and compositions of the present invention. The probiotic organisms will exhibit  
30 some or all of the following criteria: an ability to: adhere to vaginal and uroepithelial cells by electrostatic, hydrophobic or specific adhesions including but not limited to a collagen binding protein;

pass through the stomach and reach the small and large intestine and urogenital tract; grow and persist in the gastrointestinal and urogenital tracts; inhibit the adhesion of urogenital pathogens including organisms which cause urinary tract infection, bacterial vaginosis (BV) and/or yeast vaginitis; coaggregate to form a balanced flora; produce acid and other substances such as hydrogen peroxide and/or bacteriocins and bacteriocin-like compounds which inhibit pathogen growth; produce biosurfactant or related by-products of growth which interfere with adhesion of pathogens to cells and materials; resist antimicrobial agents, such as nonoxynol-9 spermicide; and/or enhance the host's immune function to further maintain a healthy urogenital flora. The orally administered lactobacilli of the present invention may be detected in a biological sample from one to about twenty-one days after intake with a molecular probe. In a preferred embodiment the biological sample is stool.

Although this invention is not intended to be limited to any particular mode of application, oral administration of the compositions are preferred. One probiotic organism may be administered alone or in conjunction with a second, different probiotic organism. In a preferred embodiment, *L. fermentum* RC-14 and *L. rhamnosus* GR-1 are administered in conjunction with each other. By "in conjunction with" is meant together, substantially simultaneously or sequentially. The compositions may be administered in the form of tablet, pill or capsule, for example. One preferred form of application involves the preparation of a freeze-dried capsule comprising the composition of the present invention. Another preferred form of application

By "amount effective" as used herein is meant an amount of probiotic organism, e.g., *Lactobacillus*, high enough to significantly positively modify the condition to be treated but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical judgment. An effective amount of *Lactobacillus* will vary with the particular goal to be achieved, the age and physical condition of the patient being treated, the severity of the underlying disease, the duration of treatment, the nature of concurrent therapy and the specific *Lactobacillus* employed. The effective amount of *Lactobacillus* will thus be the minimum amount which will provide the desired attachment to epithelial cells. The presence of about  $1 \times 10^9$  bacteria, as viable or non-viable whole cells, in 0.05 ml solution of phosphate buffered saline solution, or in 0.05 ml of suspension of agar, or the dry weight equivalent of cell wall fragments, is effective when administered in quantities of from about 0.05 ml to about 20 ml.

intravenous (where non-viable), or suppository (vaginal or rectal) routes. Depending on the route of administration, the active ingredients which comprise probiotic organisms may be required to be coated in a material to protect said organisms from the action of enzymes, acids and other natural conditions which may inactivate said organisms. In order to administer probiotic organisms by other than parenteral administration, they should be coated by, or administered with, a material to prevent inactivation. For example, probiotic organisms may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DFP) and trasylol. Liposomes include water-in-oil-in-water P40 emulsions as well as conventional and specifically designed liposomes which transport lactobacilli or their by-products to the urogenital surface.

The probiotic organisms may also be administered parenterally or intraperitoneally. Dispersions can also be prepared, for example, in glycerol, liquid polyethylene glycols, and mixtures thereof, and in oils.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, vegetable oils and

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administration, the probiotic organisms may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

- 5 Compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains about  $1 \times 10^9$  viable or non-viable e.g., lactobacilli per ml.

- 10 The tablets, troches, pills, capsules, and the like, as described above, may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as  
15 peppermint, oil or wintergreen or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid  
20 carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules or lactobacilli in suspension may be coated with shellac, sugar or both.

- 25 A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be  
30 pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the probiotic organism may be incorporated into sustained-release preparations and formulations.

It is especially advantageous to formulate oral and parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units  
5 suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of the probiotic organisms calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel  
10 dosage unit forms of the invention are dictated by and directly depending on (a) the unique characteristics of the probiotic organism and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such probiotic for the  
15 establishment and maintenance of a healthy urogenital flora.

The probiotic organism is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically or food  
20 acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in an amount approximating  $10^9$  viable or non-viable, e.g., lactobacilli, per ml. In the case of compositions containing supplementary  
25 ingredients such as prebiotics, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In one embodiment of the present invention one or more probiotic organisms in unit dosage form are  
30 administered once daily. In another embodiment of the present invention one or more probiotic organisms in unit dosage form are administered twice daily. The present invention also contemplates administration of probiotic

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compositions for a period of about one week to about thirty weeks. A preferred course of probiotic therapy contemplated by the present invention is from about one week to about eight weeks. A most preferred course of  
5 probiotic therapy contemplated by the present invention is from about one week to about four weeks. However, it is also contemplated by the present invention that the probiotic therapy of the present invention could continue throughout the life of a woman to maintain a healthy  
10 urogenital flora.

The pharmaceutically acceptable carrier may be in the form of milk or portions thereof including yogurt. Skim milk, skim milk powder, non-milk or non-lactose containing products may also be employed. Another  
15 pharmaceutically acceptable carrier contemplated by the present invention is calcium carbonate. The skim milk powder is conventionally suspended in phosphate buffered saline (PBS), autoclaved or filtered to eradicate proteinaceous and living contaminants, then freeze dried  
20 heat dried, vacuum dried, or lyophilized.

Some other examples of substances which can serve as pharmaceutical carriers are sugars, such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as  
25 sodium carboxymethylcellulose, ethylcellulose and cellulose acetates; powdered tragacanth; malt; gelatin; talc; stearic acids; magnesium stearate; calcium sulfate; calcium carbonate; vegetable oils, such as peanut oils, cotton seed oil, sesame oil, olive oil, corn oil and oil  
30 of theobroma; polyols such as propylene glycol, glycerine, sorbitol, manitol, and polyethylene glycol; agar; alginic acids; pyrogen-free water; isotonic saline; cranberry extracts and phosphate buffer solution; skim milk powder;

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**EXAMPLE 1**

Orally ingested lactobacilli traversed the gastrointestinal tract and reached and colonized the vagina.

5           Each morning and last thing at night for 14 days, ten women swallowed a probiotic solution containing >10<sup>9</sup> *L. rhamnosus* GR-1 and *L. fermentum* RC-14 suspended in 3 ml sterilized skim milk (stored at -20°C). These organisms were selected on the basis of their production  
10 of various antagonistic factors against urogenital pathogens (Reid (1999) Appl. Environ. Microbiol., 65: 3763-3766, incorporated herein by reference), including biosurfactants which inhibit adhesion of Gram positive cocci including enterococci, staphylococci and Group B  
15 streptococci, and Gram negative rods including coliforms and *Gardnerella*. The patients provided urine and vaginal swabs pre-treatment and 1, 2, 3 and 4 weeks after commencement of the therapy. Strains GR-1 and RC-14 were identified by colony and Gram stain morphology and  
20 molecular typing (Zhang, et al. (1998) Appl. Environ. Microbiol., 64:2418-2423). During therapy, patients refrained from ingestion of any other probiotic or probiotic compound.

          The patients were followed for up to 3 months.  
25 Vaginal swabs taken prior to therapy confirmed patients were free from current infection but had depleted lactobacilli numbers. After therapy, strains GR-1 and RC-14 were recovered from the vagina on the first three weeks following oral ingestion, as confirmed by culture and  
30 morphology as well as genomic fingerprinting using PCR amplified ribosomal RNA spacers.

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**TABLE 1**

			Presence of lactobacilli and identification of GR-1 and RC-14: Week of Swab Collection Post Start of Therapy on Day 1					
Patient	1 yr. history	Preswab	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12
CK	RYV	No Lacto.	++ GR-1	++ GR-1	++ GR-1	++ GR-1, RC-14	++ GR-1	++ GR-1
TR	RYV, UTI	Low Lacto.	+ GR-1	++ GR-1	NS	++ RC-14	++ GR-1	++ GR-1, RC-14
SH	RYV	No Lacto.	+ GR-1	+++ GR-1, RC-14	++ RC-14	++ Ant RC-14		
BC	RBV	Low. Lacto.	+ RC-14	++ GR-1	++ RC-14	+		
AD	RYV	Lacto.	+ GR-1	+ GR-1	+ GR-1	++ GR-1		
AC	RYV	Lacto.	+ RC-14	+	NS	++ RC-14		
SB	RBV, RYV	Lacto.	+ RC-14	+ RC-14	+ RC-14			
SO	RYV	Lacto.	++ GR-1	++ GR-1	++ GR-1, RC-14	++ GR-1, RC-14		
JA	UTI, RYV	Lacto.	++ <sup>a</sup>	++ <sup>a</sup>	++ <sup>a</sup>	++ <sup>a</sup>	++ <sup>a</sup>	++ <sup>a</sup>
AL	UTI, RYV	Lacto	+ RC-14	NS	NS	NS		

Legend:

- 5 RYV = recurrent yeast vaginitis; RBV= recurrent bacterial vaginosis; UTI = recurrent urinary tract infections in past year; No Lacto = MRS agar plate culture isolated no lactobacilli; Low Lacto = less than 1- colonies at zero dilution; +, ++, +++ = 1, 2, or 3 *Lactobacillus* isolated
- 10 by colony morphology and Gram stain; GR-1, RC-14 = identification of GR-1 or RC-14 by colony and Gram stain morphology; and/or molecular typing; Ant = patient

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**EXAMPLE 2**

Lactobacilli were rapidly detected in stool and  
5 vaginal specimens via intergenic 16S-23S Ribosomal spacer  
PCR analysis using specific primers of *L. fermentum* RC14.  
The following method was employed:

Lactobacilli isolates were cultured at 37°C for  
48 hours on an LBS plate in anaerobic chamber. One loop  
10 of bacteria colonies was picked from the LBS plate and  
suspended in 1 ml of d<sub>2</sub>H<sub>2</sub>O, then centrifuged for 1 min at  
12,000 rpm. 200 µl of InstaGene matrix (Bio-Rad) was  
added to the pellet and incubated at 56°C in a water bath  
for 30 min. The pellet was vortexed at high speed for 10  
15 seconds keeping the sample in the boiling waterbath for 8  
min. The sample was vortexed at high speed again and spun  
at 12,000 rpm for 3 min. The chromosomal DNA was stored  
at -20°C until used.

Optimal PCR conditions for different strains of  
20 *Lactobacillus* were established by using two universal  
primers from *E. coli*. The DNA fragment containing the  
spacer regions between 16S rRNA and 23S rRNA genes of RC-  
14 strains was amplified by using PCR with two universal  
primers A1 and B1 from *E. coli*. The 5' primer,  
25 5'AGTCGTAACAAGGTAAGCCG3' (SEQ ID NO:1) corresponds to a  
conserved sequence motif from the 3' end of 16S rRNAs  
[Primer A1, position 1493 - 1513 (*Escherichia coli* 16S  
rRNA numbering)] and the 3' primer, 5'C T/C A/G T/C  
TGCCAAGCATCCACT3' (SEQ ID NO:2) was deduced from an  
30 alignment of the 13 23S 5' sequences [primer B1, position  
23 - 43 (*Escherichia coli* 23S rRNA numbering)],  
respectively. DNA templates (1.6 ug, 40 µl) were

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amplified in a 100 µl reaction volume that contained 2.5 u  
Taq polymerase (Boehringer Mannheim), 100 ng of each of  
the primers, 4 mM MgCl<sub>2</sub>, 0.2 mM of each of the four dNTPs  
(Pharmacia Biotech), 10 mM Tris-Cl (PH 8.0), 50 mM KCl and  
5 1% (v/v) Triton X-100. Reaction mixtures were overlaid  
with 100 µl mini oil (liquired paraffin, VWR) and  
preheated at 95° for 5 min. Amplification was carried out  
in a AMPLITRON II Thermolyne for 40 cycles. Each  
amplification cycle was as follows: 30 seconds at 95°C  
10 (denaturation), 1 min. at 40°C, 45°C or 50°C. The optimal  
annealing temperature was 40°C for RC-14, and 1 min at  
72°C (extension). Post dwell 7 min. at 72°C. Controls  
were included in each set of amplifications. The controls  
consisted of a reaction mixture with no DNA template  
15 added.

Analysis of the degree and the specificity of  
PCR products was conducted by 2.5% agarose gel in 1x TAE  
buffer, running at 70 Volts for 2½ hours. The gel was  
stained with ethidium bromide and photographed under UV  
20 light. DNA fragment sizes were compared with the 100bp  
DNA Molecular Weight (Gibco-Life Tech.) There were two  
PCR bands for RC14 (Band 1: 220bp and Band 2: 180bp).

A QIAquick Gel Extraction Kit (Qiagen,  
Mississauga, Ontario) for extraction of DNA fragments  
25 70bp-10kb from standard agrose gel in TAE or TBE buffer  
was used to purify PCR bands.

Each of the two PCR DNA fragment bands were  
excised from the agarose gel with a scalpel and the gel  
slice was weighed. The protocol of QIAquick Gel  
30 Extraction Kit was then followed. The Kit system combined  
the spin-column with the silica-gel membrane. The DNA

band was dissolved completely with solubilization buffer  
in 50°C for 10 min. DNA adsorbed to the silica membrane  
in the high salt conditions. Pure DNA was eluted with  
Tris buffer (PH 8.0). This pure PCR product was stored at  
5 -20°C for later use.

Each PCR band product was ligated into pGEM-T  
vector (Promega). Each pGEM-T vector was transformed into  
E. coli JM 109 high efficiency competent cells by using  
Transformation Aid (MBI Fermentas Inc.) on the LB plate  
10 with 50 ug/ml ampicillin. Several white colonies or light  
blue colonies were selected as positive colonies which  
contained the PCR insert. Colonies were cultured on the  
LB-ampicillin plate. Each plate contained 32 different  
colonies. Colonies were cultured with LB-ampicillin  
15 broth. One part of culture was frozen quickly by using  
liquid nitrogen and was kept at -80°C. Another part of  
culture was used for further miniprep of plasmid DNA. The  
remainder of culture was kept at 4°C.

The QIAprep Spin Miniprep Kit (Qiagen,  
20 Mississauga, Ontario) was used to prepare plasmid DNA.  
Each of two PCR products was automatically sequenced by  
using T7 & SP6 promoter primers with two directions.  
Analysis of sequence was performed using the sequence  
analysis software package - DNA Star program.

25 DNA templates (1.6 ug, 40 µl) were amplified in  
a 100 µl reaction volume that contained 2.5 u Taq  
polymerase (Boehringer Mannheim), 100 ng of each of the  
primer, 4 mM MgCl<sub>2</sub>, 0.2 mM of each of the four dNTPs  
(Pharmacia Biotech), 10 mM Tris-Cl (PH 8.0), 50 mM KCl,  
30 and 1% (v/v) Triton X-100. Reaction mixtures were  
overlaid with 100 µl mini oil and preheated at 95°C for 5  
min. Amplification was carried out in a AMPLITRON II

Thermolyne for 25 cycles. Each amplification cycle was as follows: 30 seconds at 95°C (denaturation), 1 min. at 60°C (annealing), and 1 min. at 72°C (extension). Post dwell 7 min. at 72°C. Controls were included in each set of  
5 amplifications. *L. acidophilus* RC-14 was identified in both stool and vaginal specimens (see Example 1 and Figure 7).

Verification and confirmation of detection of  
*Lactobacillus fermentum* RC-14 was performed using a  
10 traditional API 50 commercial biochemistry test (API Systems, La Balme, Les Grottes, France) and PCR primer. Organisms were isolated from stool following 10 days of oral intake of the probiotic organism in skim milk suspension (TABLE 3).

15

TABLE 3			
Patient	Day of isolation	API50	Molecular Probe
TO	7	RC-14	RC-14
TO	14	RC-14	RC-14
DR	7	RC-14	RC-14
FH	7	RC-14	RC-14

**EXAMPLE 3**

This example illustrates the extent to which biofilm formation, undetected by most conventional  
5 diagnostic systems, can occur in the vagina and thereby seed and infect the bladder. Furthermore, the example illustrates how oral ingestion of lactobacilli, selected for their proven ability to interfere with the adhesion and growth of pathogens, can allow the host to restore a  
10 normal urogenital biofilm, thereby reducing the signs and symptoms of infection and restoring a healthy flora, comprising the patient's own lactobacilli as well as those ingested.

A 48 year old woman presented with a four year  
15 history of chronic symptomatic UTI which caused constant and often severe suprapubic pain, frequency, urgency and dysuria. Conventional laboratory culture of her urine was repeatedly reported as negative, and several specialist clinics had proposed treatments as varied as removal of  
20 the uterus, removal of the sigmoid colon and urethral stretching, all of which were refused by the patient. Careful urinalysis by the inventor showed 1,000 colony forming units of *Enterococcus faecalis*, and examination of the sloughed transitional bladder cells of the patient  
25 showed heavy colonization with a mean of 28 enterococci per each of 50 cells.

The patient orally received one vial of probiotic containing  $>10^9$  *L. rhamnosus* GR-1 and *L. fermentum* RC-14 suspended in 3 ml sterilized skim milk (stored at  $-20^{\circ}\text{C}$ )  
30 each morning and another last thing at night for 14 days. The patient provided urine and vaginal swabs on Days 6, 15 and 21, 28 and 39 for culture and identification of

lactobacilli, uropathogens and yeast. Strains GR-1 and RC-14 were identified by morphology on agar plate and under Gram stain microscopy, as well as molecular typing by genomic fingerprinting of GR-1 and RC-14 using PCR amplified ribosomal RNA spacers (i.e., a molecular probe) (see Example 2). Versalovic, et al. (1991) Nucl. Acids Res. 19:6823-31 and Versalovic, et al. (1993) J. Infect. Dis. 167:850-856 plus Zhong, et al. (1998) Appl. Environ. Microbiol. 64:2418-2423, incorporated herein by reference.

10

**TABLE 4**

ANALYSIS	DAY -6	DAY 6	DAY 15	DAY 21
Urine culture	1,000 enterococci/ml	No sample	No bacteria recovered	No bacteria recovered
Uroepithelial cell count*	28 enterococci per cell	No sample	Insufficient cells to test	0 enterococci per cell
Vaginal culture	200,000 enterococci/ml 1,000,000 long stringy indigenous <i>L. brevis</i> ; Yeast cells present	0 enterococci/ml 23,000/ml regular rod shaped indigenous <i>L. brevis</i> ; No yeast present	** sample not reliable for enumeration due to shipment problem; but, some enterococci present; indigenous and GR-1/RC-14 isolated; Yeast cells present	10,000 enterococci/ml 50,000/ml lactobacilli including GR-1/RC-14; Yeast cells present
Vaginal cells on wet mount	>100 enterococci per field of view at x1000 microscopy (score 4)	<10 enterococci per field of view (score 1)	<10 enterococci per field of view (score 1)	<10 enterococci per field of view (score 1)
Symptoms	Constant (every day) suprapubic pain, frequency, urgency, fatigue, for 4 years	Several days pain-free and noticeably less frequency, urgency and fatigue	Several days pain-free and noticeably less frequency, urgency and fatigue	Most days pain-free and noticeably less frequency, urgency and fatigue

\* uroepithelial cells sloughed and present in mid-stream urine were collected, Gram stained and examined under light microscopy

\*\* The total *Lactobacillus* viable count from vaginal culture on Day 28 was 1,500,000 and on Day 39 was 300,000 colony forming units per ml.

20

It was determined that two probiotic strains survived stomach acid and bile, and migrated to the vaginal mucosa where they colonized. In addition, the  
5 enterococci, which were seeding the bladder from their heavy biofilm presence in the urogenital tract, became depleted after only six days probiotic therapy and were subsequently eradicated from the bladder and  
10 significantly reduced in the vagina within two to three weeks. The oral probiotic treatment alleviated the patient's symptoms, eradicated the urinary tract infection and restored a healthy urogenital flora within three weeks.

These experiments show, for the first time,  
15 that probiotic lactobacilli can be delivered to the vagina, colonize and restore a healthy flora by oral intake.

### EXAMPLE 4

Strains of *Lactobacillus* species found in the vagina of healthy women, namely, *L. rhamnosus*, *L.*

5 acidophilus, *L. crispatus*, *L. fermentum*, *L. plantarum*, *L.*  
*casei*, *L. paracasei*, *L. jensenii*, *L. gasseri*, *L.*  
*cellobiosus*, *L. brevis*, *L. delbrueckii*, *L. rogosae*, *L.*  
*bifidum*, with properties such as those possessed by GR-1,  
RC-14 and B-54 or other defined strains with properties  
10 identified previously (Reid & Bruce, 1998) can colonize  
the vagina following oral ingestion. Evidence of this  
was found in a 37 year old woman whose stool and vagina  
contained the same strain of *L. paracasei* spp. *paracasei*.

This result further verifies that the intestinal tract  
15 is the source of *Lactobacillus* in the urogenital tract  
and therefore oral ingestion can lead to *Lactobacillus*  
strains colonizing the intestinal and urogenital tracts,  
as demonstrated in Figures 1 and 2 and Examples 1 and 3.





**EXAMPLE 6**

**Bacterial strains:** *Lactobacillus rhamnosus* GG, *L. rhamnosus* GR-1, *L. fermentum* RC-14, were grown in MRS broth in anaerobic conditions at 37°C for 1-2 days. The following pathogens were examined: type strains of *Gardnerella vaginalis*, *Candida tropicalis* and *C. albicans*. These were grown in enriched media overnight at 37°C.

**Inhibition of pathogen growth:** An agar overlay technique was used as described previously (McGroarty, et al. (1988) Can. J. Microbiol., 34:974-978, incorporated herein by reference), whereby *L. rhamnosus* GR-1 or *L. fermentum* RC-14 were grown in a bottom layer of agar, a top layer was then added with pathogens, and zones of inhibition were noted after 24 hours.

**Inhibition of pathogen adhesion:** Experiments were designed to determine the extent to which GR-1, RC-14, two commercial and two freshly isolated lactobacilli inhibited adhesion of marker pathogens (enterococci) and two intestinal/genital organisms group B streptococci and *Gardnerella* lactobacilli in vitro. Lactobacilli biosurfactants were isolated (Reid, et al. (1999) Methods Enzymol, 310:426-432, incorporated herein by reference) adsorbed onto polystyrene plates and challenged with various pathogens.

Both *L. rhamnosus* GR-1 and *L. fermentum* RC-14 inhibited adhesion of *E. faecalis* and *Streptococcus* and *L. fermentum* RC-14 inhibited adhesion of *G. vaginalis* (Table 5).

[illegible][illegible]

EXAMPLE 7

Randomized, placebo-controlled study to examine health outcomes in healthy women. Sixty four healthy women were randomly allotted to receive either a freeze-dried capsule containing *L. rhamnosus* GR-1 and *L. fermentum* RC-14 or calcium carbonate placebo by mouth once daily for 60 days. The subjects and investigators were blinded to therapy. Two vaginal swabs were collected at days 0 (before treatment), 7, 35, 56 and 90: one was cultured for total lactobacilli, yeast and coliforms, and the other was Gram stained and given a Nugent score (Nugent, et al. (1991) J. Clin. Microbiol, 29:287-301, incorporated herein by reference). Upon completion of the study, the patients were asked if there had been a noticeable improvement in their quality of life and vaginal well-being during the treatment.

In the randomized, placebo-controlled study of healthy subjects, there was considerable evidence of improved well being: 30% women reported improved vaginal health on lactobacilli compared to 12% with placebo (Fisher's exact test,  $p=.168$ ). All other subjects reported no change, although two on placebo felt worse than before. No adverse side effects were reported. The Nugent scores for the subjects showed an improvement by day 56 (from Bacterial Vaginosis to normal or intermediate) in 37% women given lactobacilli compared to 13% controls ( $p=.021$ ). The lactobacilli oral therapy provided a significant impact on the vaginal flora by decreasing the yeast and enteric pathogen counts (Figs 7A, 7B).

The study demonstrated that probiotic *L. rhamnosus* GR-1 and *L. fermentum* RC-14 confer health benefits to the vagina of women. This conclusion is drawn from *in vitro* inhibition of pathogen growth and adhesion,

and clinical data. The inhibition is not due to hydrogen peroxide, as RC-14 produces hydrogen peroxide, while GG does so weakly and GR-1, Shirota and DN-114 001 do not produce hydrogen peroxide.

- 5           Although the lactobacilli did not inhibit *Candida* growth *in vitro*, the GR-1/RC-14 therapy reduced candida colonization in the vagina.

- Given the high incidence of yeast vaginitis (Ferrer, J. (2000) Int. J. Gynaecol. Obstet., 71 (Supp. 1):21-27, incorporated herein by reference) this  
10       invention provides an effective therapy to treat yeast vaginitis. Combined with lactobacilli inhibition of adhesion of group B streptococci and *Gardnerella* and uropathogens adhesion (Valraeds, et al. (1998) J. Med. Microbiol., 49:790-784), the GR-1/RC-14 combination of  
15       the present invention has properties effective to restore and maintain the normal vaginal flora, and reduce the risk of complications such as preterm birth.

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**EXAMPLE 8**

**Randomized, placebo controlled study to determine effects of *L. fermentum* RC-14/*L. rhamnosus* GR-1 therapy on vaginal flora health.**

5

**Lactobacillus strains.** *L. rhamnosus* GR-1 and *L. fermentum* RC-14 were grown in MRS broth (Sigma, Detroit), tested for purity, and freeze dried into gelatin capsules in dosage forms of  $8 \times 10^8$  (Group 1),  $6 \times 10^9$  (Group 3) and two per day of the  $8 \times 10^8 = 1.6 \times 10^9$  (Group 2). Commercially available capsules containing  $10^{10}$  viable *L. rhamnosus* GG (Group 4) were purchased. No significant loss of viability was found during the duration of the study.

15 **Subjects and randomization.** Forty-two healthy subjects signed an Informed Consent using a format approved by the Ethics Review Board of The University of Western Ontario. The inclusion criteria were for healthy women, 17 and over (actual range was 17-50 with mean of  $31 \pm 8$ ), with or  
20 without a past history of urogenital infections (UTI, BV or yeast vaginitis) in the past 5 years, or who had been on long term, low dose antibiotics for UTI. The exclusion criteria were patients with abnormal renal function (serum creatinine  $>110 \mu\text{mol/l}$ , upper limit  
25  $90 \mu\text{mol/l}$ ) or pyelonephritis, women who were pregnant, women who were lactose intolerant or receiving prednisone, immunosuppressive drugs, antimicrobial therapy or were using nonoxynol-9 as a spermicide agent.

30 The capsules were dispensed in a randomized manner, and researchers were blinded as to the treatments given. Patients given twice daily therapy were aware of their regimen, as was a nurse.

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**Sampl Processing.** Deep vaginal swabs were collected within two days prior to study, then on days 7, 14, 21, 28, 35, and 41. The swabs were placed onto glass slides and examined using the Nugent Scale (0-3 normal; 4-6 intermediate; 7-10 BV). (Nugent, et al. (1991) J. Clin. Microbiol, 29:297-301, incorporated herein by reference).

**Results.** While all the subjects reported feeling normal with respect to the urogenital tract, only 17/42 (40%) actually showed a normal Nugent score prior to commencement of therapy. BV was diagnosed by Nugent scoring in 4/10 women in Group 1, 3/12 in Group 2, 4/11 in Group 3 and 3/9 in control Group 4 (Table 6). BV scores reverted to normal or intermediate at day 28 in 7/11 (64%) subjects given one of three forms of GR-1/RC-14 therapy. At two week follow-up, 2/9 (22.2%) patients in the GG control Group 4 had a BV score, compared to 4/29 (13.8%) in the GR-1/RC-14 treated group ( $p=.613$  Fisher's Exact Test).

Compliance was excellent and all 42 subjects completed the study. As shown in Table 7, the control GG group showed no improvement in the number of subjects with normal vaginal flora at the end of 28 days treatment. On the contrary, treatment with the GR-1/RC-14 combination twice daily (still almost one log less than one dose of the GG control) resulted in 50% more normal scores than before treatment started.

Within two weeks of completion of treatment, the vaginal flora remained normal in 90% of women given the twice daily GR-1/RC-14 lactobacilli, and this was significantly better than the GG control ( $p=.017$ ) Of women with a history of urogenital infections at the

start of treatment, only those given GR-1/RC-14 showed consistently improved normal flora at the end of treatment.

Of the subjects who had a history of yeast vaginitis an additional 7/25 (28%) converted to a normal flora with GR-1/RC-14 treatment, while one (13%) of the GG controls converted. Of the five subjects who had a history of BV, one (#128) converted to a normal flora with GR-1/RC-14 treatment, while none of the GG controls converted. None of the patients reported symptomatic yeast vaginitis, UTI or BV during the 6 week test period.

The pool of subjects was quite typical of healthy women in a fairly well educated, middle class community, and not those at high risk of sexually transmitted diseases studied elsewhere (Schwebke, et al. (1999) J. Infect. Dis., 180:1632-1636). Their overall history of bladder and vaginal infection was not unexpected, given the high incidence of these diseases amongst women. The low incidence of a history of BV was also not surprising, given its poor diagnosis in community clinics. The prevalence of normal flora at 40% at the start of the study was lower than the 76.5% reported by Schwebke.et al. (supra), perhaps illustrating the innate abnormality of women with a history of urogenital infections and antibiotic treatment.

The finding that the flora was normal in the majority of subjects after treatment with the combination of lactobacilli strains GR-1 and RC-14, and not the control patients taking *L. rhamnosus* GG, indicated that the two strain combination therapy had an impact on the flora. The probiotic therapy restored the vaginal flora to normal in 90% in one group of patients, bringing it above the levels reported for healthy women (Id.). In

subjects who started with a normal flora, 12/13 (92%) retained normality at day 28 and one scored intermediate following GR-1/RC-14 therapy. This is almost double the 48% of women found elsewhere to maintain a normal flora over a menstrual cycle as determined by Nugent scoring (Keane, et al. (1997) STD AIDS, 8:489-494). The present invention therefore contemplates daily probiotic use as a means to maintain a healthy urogenital flora.

There was no significant difference between Group 1 and the control at day 28 or day 42, suggesting that a daily dose greater than  $10^9$  is required. The fact that the twice a day dose was better than the control was not due to the former being higher than the latter. On the contrary, the twice daily dose was still considerably fewer organisms than those in the control product. The results for *L. rhamnosus* GG are consistent with its inability to colonize the vagina or protect the host from recurrence of UTI (Kontiokari, et al. (2000) 38<sup>th</sup> Annual Meeting of Infectious Diseases Society of America, New Orleans-Abstract).

The prevalence of bacterial vaginosis was 26% (11/42) at study commencement. BV, as diagnosed by Nugent Gram stain scoring, appeared to be eradicated in some subjects by oral treatment with the combination of GR-1 and RC-14.

The GG control did not improve the vaginal flora in patients prone to recurrence of UTI, whereas treatment with all three doses of GR-1/RC-14 showed significant improvement and maintenance of a normal flora.



**Table 6.** Patients were given  $8 \times 10^8$  (Group 1 - #104-133), two per day of  $8 \times 10^8 = 1.6 \times 10^9$  (Group 2 - #101-145) and once per day  $6 \times 10^9$  (Group 3 - 114-132) of *L. rhamnosus* GR-1 and *L. fermentum* RC-14.

**Nugent Scoring Outcomes**

Patient #	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
104	YV	BV	BV	BV	BV	BV	BV	N/A
110	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
111	YV	Int.	Int.	Int.	Int.	Normal	Normal	Int.
113	UTI	Int.	Normal	Int.	Int.	Int.	BV	Int.
116	UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
117	UTI/YV/BV	Normal	Normal	Int.	Normal	Normal	Normal	Normal
118	YV	BV	Normal	Normal	Normal	Normal	Normal	Normal
120	YV	BV	Int.	Int.	Int.	Int.	Int.	BV
125	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
133	None	BV	BV	BV	BV	BV	BV	BV
101	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
102	YV	Int.	Int.	Normal	Normal	Normal	Int.	Normal
103	UTI	Int.	Normal	Normal	Int.	Normal	Normal	Normal
105	YV/UTI	BV	BV	Int.	Int.	Int.	Normal	Normal
106	YV/UTI	BV	BV	Int.	Normal	Normal	Normal	Normal
107	YV/BV	Normal	Normal	Int.	N/A	Int.	Int.	Int.
108	UTI	BV	BV	Int.	N/A	Int.	N/A	N/A
109	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
121	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
139	YV	Int.	Int.	Int.	Normal	Normal	Normal	Normal
143	BV/YV	Normal	Normal	Int.	Normal	Normal	Normal	N/A
145	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
114	UTI/YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
119	UTI/YV	Int.	Int.	Int.	Int.	Normal	Int.	Int.
123	YV	Normal	Normal	Normal	Normal	Normal	N/A	Normal
124	YV	BV	BV	Int.	Int.	BV	BV	BV
126	None	Int.	BV	Int.	Int.	Int.	N/A	Int.
127	YV	Int.	Int.	Int.	Int.	Int.	Int.	Int.
128	UTI/BV/YV	BV	BV	N/A	Int.	Normal	Int.	Int.
129	UTI/BV/YV	Normal	Normal	Normal	Normal	Normal	Normal	Int.
130	UTI	BV	BV	BV	Int.	Int.	Int.	N/A
131	YV	Int.	Int.	Normal	Int.	Int.	Int.	Normal
132	None	BV	BV	BV	BV	BV	BV	BV

5 **None** - no past history of urogenital infection; **BV** - asymptomatic bacterial vaginosis and scores within the BV Nugent

range of 7-10; **YV** - yeast vaginitis; **UTI** - urinary tract infection; **Normal** - scores within the normal Nugent range of 0-3;

10

**Int.** - scores within the intermediate Nugent range of 4-6; **N/A** - not available

**Table 7.** Patients in Group 4 given once daily  $10^{10}$  *L. rhamnosus* GG as control.

Nugent Scoring Outcomes								
Patient #	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
134	UTI/YV	Normal	Int.	Int.	Normal	Int.	Int.	Int.
135	YV	BV	BV	N/A	N/A	Int.	Int.	Int.
136	BV/YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
137	UTI/YV	Int.	Int.	Int.	Normal	Int.	Int.	BV
138	YV	BV	BV	BV	Normal	Normal	Normal	Normal
140	UTI/YV	Int.	Int.	Int.	Int.	Int.	Int.	Int.
141	None	BV	BV	BV	N/A	N/A	N/A	BV
142	YV/BV	Normal	Int.	Normal	Normal	Normal	Normal	Normal
144	YV	Normal	Normal	Normal	Int.	Int.	Int.	Int.

**None** - no past history of urogenital infection; **BV** - asymptomatic bacterial

- 5 **vaginosis and scores within the BV Nugent range of 7-10; YV - yeast vaginitis; UTI - urinary tract infection; Normal - scores within the normal Nugent range of 0-3; Int. - scores within the intermediate Nugent range of 4-6; N/A - not available**